Obesity susceptibility loci and dietary intake in the Look AHEAD Trial1–3


ABSTRACT

Background: Genome-wide association studies (GWAS) have identified consistent associations with obesity. However, the mechanisms remain unclear.

Objective: The objective was to determine the association between obesity susceptibility loci and dietary intake.

Design: The association of GWAS-identified obesity risk alleles (FTO, MC4R, SH2B1, BDNF, INSIG2, TNNI3K, NISCH-STAB1, MTIF3, MAP2K5, QPCTL/GIPR, and PPARG) with dietary intake, measured through food-frequency questionnaires, was investigated in 2075 participants from the Look AHEAD (Action for Health in Diabetes) clinical trial. We adjusted for age, sex, population stratification, and study site.

Results: Obesity risk alleles at FTO rs1421085 significantly predicted more eating episodes per day (P = 0.001)—an effect that persisted after adjustment for body weight (P = 0.004). Risk variants within BDNF were significantly associated with more servings from the dairy product and the meat, eggs, nuts, and beans food groups (P ≤ 0.004). The risk allele at SH2B1 rs4788099 was significantly associated with more servings of dairy products (P = 0.001), whereas the risk allele at TNNI3K rs1514176 was significantly associated with a lower percentage of energy from protein (P = 0.002).

Conclusion: These findings suggest that obesity risk loci may affect the pattern and content of food consumption among overweight or obese individuals with type 2 diabetes. The Look AHEAD Genetic Ancillary Study was registered at clinicaltrials.gov as NCT01270763 and the Look AHEAD study as NCT00017953. Am J Clin Nutr 2012;95:1477–86.

INTRODUCTION

Obesity is a major public health problem associated with an increased risk of cardiovascular disease (1). Obesity susceptibility loci identified through genome-wide association studies (GWAS)1 and replicated in multiple independent cohorts have provided new insights into the genetic factors that contribute to the development of obesity. The fat mass and obesity-associated gene is associated with preference for energy-dense food (13), greater consumption

Many of these genes are expressed in the brain, particularly in the feeding centers of the hypothalamus, emphasizing the role of the central nervous system and potentially dietary intake in obesity predisposition (8). Studies in children indicated that the obesity risk allele at rs9939609 in the FTO gene is associated with preference for energy-dense food (13), greater consumption

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2 Look AHEAD genetic ancillary study funding and support (U01DK050692-11S1 and DK072497). Look AHEAD is supported by the Department of Health and Human Services through the following cooperative agreements from the NIH: DK57136, DK57149, DK56990, DK57177, DK57171, DK57151, DK57182, DK57131, DK57002, DK57078, DK57154, DK57178, DK57219, DK57008, DK57135, and DK56992. The following federal agencies have contributed support: National Institute of Diabetes and Digestive and Kidney Diseases (Barbara Harrison, Van S Hubbard, and Susan Z Yanovski); National Heart, Lung, and Blood Institute (Lawton S Cooper, Jeffrey Cutler, and Eva Obarzanek); National Institute of Nursing Research; National Center on Minority Health and Health Disparities; Office of Research on Women’s Health; and the CDC (Edward W Gregg, David F Williamson, and Ping Zhang). Additional support was received from The Johns Hopkins Medical Institutions Bayview General Clinical Research Center (M01RR02719); the Massachusetts General Hospital Mallinckrodt General Clinical Research Center (M01RR01066); the University of Colorado Health Sciences Center General Clinical Research Center (M01RR00051) and Clinical Nutrition Research Unit (P30 DK48520); the University of Tennessee at Memphis General Clinical Research Center (M01RR0021140); the University of Pittsburgh General Clinical Research Center (M01RR000564); NIH grants (DK 046204 and DK072497); the University of Washington/Va Puget Sound Health Care System Medical Research Service, Department of Veterans Affairs; and Frederic C Barter General Clinical Research Center (M01RR01346). The following organizations have committed to make major contributions to Look AHEAD: Federal Express; Health Management Resources; Johnson & Johnson, LifeScan Inc; Optifast-Novartis Nutrition; Roche Pharmaceuticals; Ross Product Division of Abbott Laboratories; Slim-Fast Foods Company; and Unilever.
of fat and calories (14), consumption of palatable food after having eaten a meal (15), and reduced satiety (16), although a lack of association between FTO rs9939609 and caloric intake or the percentage of energy from fat has also been reported (17). In adult populations, MC4R obesity risk alleles were shown to be associated with a greater caloric intake and a greater percentage of energy from fat in one study of women from the Nurses’ Health Study (18) but not in a second sample of men and women of Scottish descent (19). Obesity risk alleles at SH2B1 were associated with greater total fat intake, SFA intake, and MUFA intake in Dutch women (20), but no associations of dietary intake with FTO or MC4R were observed.

The goal of the current study was to determine the association of obesity risk single nucleotide polymorphisms (SNPs) identified through GWAS (within or in the regions of FTO, SH2B1, BDNF, INSIG2, TNNI3K, NISCH-STAB1, MTIF3, MAP2K5, QPCTL/GIPR, MC4R, INSIG2, and PPARG) and available on the ITMAT-Broad-CARe array chip (IBC) (21) or through additional genotyping with measures of dietary intake in the Look AHEAD (Action for Health in Diabetes) cohort—a sample of ethnically diverse overweight and obese participants with type 2 diabetes.

SUBJECTS AND METHODS

Participants

The design and methods of the Look AHEAD trial have been reported elsewhere (22), as have the baseline characteristics of the randomized cohort (23). Of the 5145 ethnically diverse overweight and obese Look AHEAD subjects with type 2 diabetes, aged 45–76 y at baseline, the first 2757 completed the dietary substudy (24); 2163 of the participants in the dietary substudy provided genetic consent and were in clinical centers participating in this ancillary study. Of the 2163, 65 were missing genetic data as a result of failed genotyping, and 23 were missing genetic data as a result of a high degree of missing genotype calls (>5%), yielding an effective sample size of 2075 (Figure 1). Participants who completed the Look AHEAD dietary substudy were somewhat younger than those who did not (57.2 ± 7.2 y compared with 60.5 ± 5.9 y; P < 0.0001) because of a change in the age inclusion criteria during year 2 of the recruitment period. No significant differences in sex, race, or educational attainment were found between the 2 groups. All participants included in this study provided written informed consent for participation in the Look AHEAD trial and genetic analyses in accordance with the requirements of the Institutional Review Board at their local institution. The current data analysis was approved by the Miriam Hospital Institutional Review Board, and the procedures that were followed were in accordance with The Miriam Hospital Guidelines.

Anthropometric measures

Weight and height were measured in duplicate by using a digital scale and a standard wall-mounted stadiometer. BMI was calculated as weight (in kg) divided by height (in m) squared.

Dietary assessment

The Look AHEAD semiquantitative, previously validated food-frequency questionnaire (FFQ) was selected to measure food and nutrient intakes (24–26). The FFQ is a modified version of the Diabetes Prevention Program Food-Frequency questionnaire and was designed to collect information about usual intake of food items during the preceding 6 mo. The Diabetes Prevention Program food list, developed for regional and ethnic sensitivity, was formed by using the IBC chip, a gene-centric 50,000-SNP array designed by the Children’s Hospital of Philadelphia (version 1.0, 2007; Children’s Hospital of Philadelphia). The data set was filtered for potentially relevant loci across a range of cardiovascular, metabolic, and inflammatory syndromes (21). The data set with 14 SNP genotypes at or near 9 reported genes was filtered for individuals with <5% missing genotypes and SNPs with <5% genotypes.

Genotyping

The genomic DNA extraction was based on the use of the FlexiGene DNA Kit (Qiagen Inc) as described by the manufacturer, and DNA quantitation was performed by using the PicoGreen dsDNA Quantitation Reagent (Invitrogen Inc). Genotyping was carried out at the Children’s Hospital of Philadelphia by using the IBC chip, a gene-centric 50,000-SNP array designed to assess potentially relevant loci across a range of cardiovascular, metabolic, and inflammatory syndromes (21). The data set with 14 SNP genotypes at or near 9 reported genes was filtered for individuals with <5% missing genotypes and SNPs with <5%
missing data. The mean genotyping success rate for the candidate SNPs was 99.6%. Two additional SNPs in the regions of 2 additional genes were genotyped with Taqman Applied Biosystems Assays-On-Demand by using an Applied Biosystems 7900HT: the MC4R polymorphism rs17782313 (Applied Biosystems catalog number C__32667060_10) and the INSIG2 rs7566605 polymorphism (Applied Biosystems catalog number C__29404113_20).

Gene and SNP selection

We searched the published literature and selected SNPs that had been associated with obesity by GWAS (2–9, 28, 29) or weight loss (10, 12) and appeared on the IBC (21) or, in the case of MC4R and INSIG2, were genotyped by Taqman. The SNP array also included Ancestry Informative SNP Markers and haplotype-tagging SNPs. GWAS obesity SNPs not on the IBC were replaced by proxies when possible by using the SNP Annotation and Proxy Search tool (30) based on haplotype maps of US residents of European ancestry (CEU) and Yoruba people of Ibadan (YRI) as follows: FTO rs9930506 was replaced by rs9922708 [distance 681 base pair (bp) r^2 = 1.00, D’ = 1.00 in both CEU and YRI], BDNF rs925946 was replaced by rs1401635 (distance 26,789 bp r^2 = 0.96, D’ = 1.00 in CEU; no proxy was available in YRI), SH2B1 rs7498665 was replaced by rs4788099 (distance 27,514 bp r^2 = 1.00, D’ = 1.00 in CEU and D’ = 1.00 and r^2 = 0.94 in YRI), TNNI3K rs1514175 was replaced by rs1514176 (distance 48 bp r^2 = 1.00, D’ = 1.00 in CEU and r^2 = 1.00, D’ = 1.00 in YRI), NISCH-STAB1 rs6784615 was replaced by rs4687617 (distance 2851 bp r^2 = 1.00, D’ = 1.00 in CEU; no proxy was available in YRI), MTIF3 rs4771122 was replaced by rs7988412 (distance 19898, r^2 = 0.83, D’ = 1.00 in CEU; no proxy was available in YRI), MAP2K5 rs2241423 was replaced by rs2241420 (distance 4022 bp, r^2 = 0.91, D’ = 1.00 in CEU, r^2 = 0.97, D’ = 1.00 in YRI), and QPCTL/GIPR rs2287019 was replaced by rs11672660 (distance 21988 bp, r^2 = 0.83, D’ = 1.00 in CEU, r^2 = 0.892, D’ = 1.00 in YRI).

Statistical analysis

Observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium by using a chi-square test in the 2 most populous racial-ethnic groups (non-Hispanic whites and African Americans). Pearson correlations were used to examine the association between dietary variables, BMI, and weight. Multivariable linear regression analyses were carried out to examine genetic associations with the dietary variables. Additive coding for the number of copies of rare alleles was used, unless the marker minor allele frequency (MAF) fell below 20%, in which case the rare genotype was combined with the intermediate genotype. Therefore, linear regression coefficients capture the effect of a single copy of the rare allele (MAF > 0.20) or of being a rare allele carrier (MAF < 0.20). In cases in which the risk allele differs from the rare allele, negating the regression coefficients gives the effect of each additional copy of the risk allele (MAF > 0.20) or of being a risk allele homozygote (MAF < 0.20).

All analyses were adjusted for age, sex, study site, and population stratification. For total caloric intake, we further considered whether any observed associations were attributable to weight in secondary models. For eating occasions and the Food Guide Pyramid food groups, we considered whether any observed associations were attributable to total caloric intake by including total caloric intake as a covariate in secondary models. A Huber-White sandwich estimator was used to produce SE robust to deviations from normality (31, 32). Principal component analysis of the genotypic correlation matrix of the 16 markers of interest suggested that the effective number of linearly independent markers in the data set was only 13 (33). Therefore, one can maintain the family-wise error rate at 0.05 via Sidak’s adjustment.
for multiplicity by declaring as statistically significant only those markers with a nominal significance level of 0.05/13 = 0.004 (34). However, because the markers were selected a priori due to their association with obesity, we also consider associations reaching a nominal threshold for statistical significance of 0.05. Analyses were performed by using Splus 8.2 for Solaris/Linux (35). The analyses were performed at Brown University.

To control for admixed study population, all IBC SNPs were examined by principal component analysis using the EIGENSTRAT algorithm (36) as implemented in Golden Helix version 7.1 (Bozeman). Results indicated that most of the variance among the Look AHEAD cohort was accounted for by the first 2 principal components, which agreed with self-reported ethnicity. Accordingly, the first 2 principal components were included as covariates in our analyses to adjust for population stratification in the multi-ethnic Look AHEAD cohort.

RESULTS

Participant characteristics of the subcohort of Look AHEAD used in these analyses are shown in Table 1. Participants came from an ethnically diverse background, and 56% were women. They had an average age of 57.6 years and a BMI in the obese range. Participants reported consuming ~2000 kcal/d over an average of 4.7 eating episodes. Forty percent of calories were derived from fat, 44% from carbohydrate, and 17% from protein.

SNP characteristics, including the obesity-risk allele identified in the prior literature, are presented in Table 2. All SNPs under study conformed to Hardy-Weinberg equilibrium, except for rs2241420 in non-Hispanic whites (P < 0.001) and rs1401635 in African Americans (P = 0.02). Given that this sample was selected for being overweight/obese and having type 2 diabetes and that the genetic markers were selected for association with obesity, we retained these markers in analyses.

The 4 FTO SNPs were in strong linkage disequilibrium in our white subsample (r² = 0.80–0.99), but differed in the degree of disequilibrium in our African American subsample (rs3751812 and rs1421085: r² = 0.96; rs3751812 and rs9922708: r² = 0.51; rs1421085 and rs9922708: r² = 0.48; rs9939609 with other SNPs: r² < 0.13). Two BDNF SNPs, rs6265 and rs10767664, were in strong linkage disequilibrium in both our white (r² = 0.79) and African American (r² = 0.69) subsamples. The third BDNF SNP, rs1401635, appeared unrelated to the first two (r² < 0.11 in both whites and African Americans).

Total caloric intake was positively associated with weight (r = 0.22, P < 0.001), BMI (r = 0.14, P < 0.001), and number of eating episodes (r = 0.17, P < 0.001). Number of eating episodes showed no association with BMI (r = 0.00, P = 0.926) or weight (r = −0.04, P = 0.108).

Total calories

Obesity risk markers within BDNF and FTO were nominally associated with greater total caloric intake (Table 3). Risk alleles at FTO rs1421085, rs3751812, and rs9922708 were associated with 57–60 more calories per day per copy (P = 0.031–0.035). Carriers of the AA genotype at BDNF rs10767664 or the GG genotype at BDNF rs6265 consumed on average >100 kcal/d more than did carriers of the less common genotypes (P = 0.006–0.007). The effect of the 3 FTO SNPs on dietary intake was diminished by statistical adjustment for weight (P = 0.066–0.068). However, BDNF rs10767664 and rs6265 remained nominally associated with total caloric intake after statistical adjustment for weight (rs6265, P = 0.007; rs10767664, P = 0.007).

Number of eating episodes

Genetic associations with number of eating episodes are presented in Table 4. Obesity risk alleles at rs1421085 within FTO were significantly associated with eating a greater number of meals and snacks per day (P = 0.001), an effect that persisted after further adjustment for total caloric intake (P = 0.004). Obesity risk alleles at FTO rs3751812, rs9922708, and rs9939609 showed similar effects in the same direction but significance was nominal (P = 0.014–0.039). For OPCTI/GIPR rs11672660, MC4R rs17782313, and PPARγ rs1801282, risk alleles associated with obesity or diabetes and resistance to weight loss in the prior literature were nominally associated with fewer eating occasions per day. Statistical adjustment for total caloric intake did not substantially alter these associations.

Percentage of energy from fat, carbohydrate, and protein

Genetic associations with percentage of energy from fat, carbohydrate, and protein are presented elsewhere (see Supplemental Table 1 under “Supplemental data” in the online issue). The obesity risk allele at rs1514176 (TNNS3K region) was significantly associated with a lower percentage of energy from protein (P = 0.002; −0.28% per copy). Each copy of the obesity risk allele at FTO rs1421085 was also nominally associated with a greater percentage of energy from fat (P = 0.019; 0.52% per

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**Table 1** Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (n = 2075)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women [n (%)]</td>
<td>1163 (56.0)</td>
</tr>
<tr>
<td>Race [n (%)]</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>324 (15.6)</td>
</tr>
<tr>
<td>American Indian/Alaskan Native</td>
<td>12 (0.6)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>23 (1.1)</td>
</tr>
<tr>
<td>White</td>
<td>1595 (76.9)</td>
</tr>
<tr>
<td>Other (multiple)</td>
<td>121 (5.8)</td>
</tr>
<tr>
<td>Ethnicity [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>161 (8.2)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>57.6 ± 7.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.3 ± 6.1</td>
</tr>
</tbody>
</table>

Dietary intake

| Total energy intake (kcal)      | 1992.1 ± 875.7 |
| Eating occasions (no./d)        | 4.7 ± 1.2      |
| Carbohydrate (% of energy)      | 43.6 ± 7.7     |
| Fat (% of energy)               | 40.0 ± 7.0     |
| Protein (% of energy)           | 17.2 ± 2.9     |
| Bread, cereal, rice, pasta       | 3.2 ± 1.7      |
| Vegetables (servings/d)         | 2.9 ± 1.5      |
| Fruit (servings/d)              | 1.9 ± 1.4      |
| Milk, yogurt, cheese (servings/d)| 2.2 ± 1.6     |
| Meat, poultry, fish, dry beans, eggs, nuts (servings/d)| 2.7 ± 1.5 |
| Fats, oils, sweets (servings/d) | 2.2 ± 1.8      |

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1 The number of American Indian participants included in this study is less than that in the parent Look AHEAD trial.

2 Mean ± SD (all such values).
copy). No association with percentage of energy from carbohydrate and no other associations between the obesity risk alleles and percentage of energy from fat or carbohydrate were observed.

**Food Guide Pyramid food groups**

Several associations of obesity risk alleles with daily servings of breads, cereals, rice and pasta (pyramid food group 1 [PFG1]); dairy products (PFG4); meats, eggs, nuts and beans (PFG5); and fats, oils, and sweets (PFG6) were observed (see Supplemental Table 2 under “Supplemental data” in the online issue). For example, risk variants within *BDNF* were significantly associated with more servings from the dairy product and the meat, eggs, nuts, and beans food groups (*P* ≤ 0.004), whereas the risk allele at *SH2B1* rs4788099 was also significantly associated with more servings of dairy products (*P* = 0.001). Statistical adjustment for total caloric intake largely diminished these associations, which suggests that any genetic associations with servings within these food groups were likely mediated via previously noted effects on total caloric intake. In the primary exception,

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Minor allele</th>
<th>β ± SE</th>
<th>P value</th>
<th>Adjusted for age, sex, study site, population stratification, and weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNN13K</td>
<td>rs1514176</td>
<td>G</td>
<td>45.193 ± 26.169</td>
<td>0.851</td>
</tr>
<tr>
<td>INSG2</td>
<td>rs7566605</td>
<td>C</td>
<td>−20.850 ± 27.815</td>
<td>0.453</td>
</tr>
<tr>
<td>PPARG</td>
<td>rs1801282</td>
<td>G2</td>
<td>97.836 ± 52.528</td>
<td>0.064</td>
</tr>
<tr>
<td>NISCH-STAB1</td>
<td>rs4687617</td>
<td>G</td>
<td>85.890 ± 61.122</td>
<td>0.160</td>
</tr>
<tr>
<td>BDNF</td>
<td>rs10767664</td>
<td>T2</td>
<td>−104.005 ± 38.290</td>
<td>0.007</td>
</tr>
<tr>
<td>BDNF</td>
<td>rs6265</td>
<td>A2</td>
<td>−107.090 ± 38.659</td>
<td>0.006</td>
</tr>
<tr>
<td>BDNF</td>
<td>rs1401635</td>
<td>C</td>
<td>−25.061 ± 26.324</td>
<td>0.341</td>
</tr>
<tr>
<td>MTIF3</td>
<td>rs7988412</td>
<td>A</td>
<td>3.427 ± 40.260</td>
<td>0.932</td>
</tr>
<tr>
<td>MAP2K5</td>
<td>rs2241420</td>
<td>A</td>
<td>8.400 ± 28.813</td>
<td>0.771</td>
</tr>
<tr>
<td>SH2B1</td>
<td>rs4788099</td>
<td>G2</td>
<td>33.762 ± 27.025</td>
<td>0.211</td>
</tr>
<tr>
<td>FTO</td>
<td>rs1421085</td>
<td>C</td>
<td>60.139 ± 27.833</td>
<td>0.031</td>
</tr>
<tr>
<td>FTO</td>
<td>rs3571812</td>
<td>A</td>
<td>58.685 ± 27.836</td>
<td>0.035</td>
</tr>
<tr>
<td>FTO</td>
<td>rs9922708</td>
<td>A</td>
<td>56.953 ± 26.736</td>
<td>0.033</td>
</tr>
<tr>
<td>FTO</td>
<td>rs9939609</td>
<td>A</td>
<td>45.604 ± 27.764</td>
<td>0.101</td>
</tr>
<tr>
<td>MC4R</td>
<td>rs17782313</td>
<td>C</td>
<td>17.953 ± 30.710</td>
<td>0.559</td>
</tr>
<tr>
<td>QPCTL/GIPR</td>
<td>rs11672660</td>
<td>A2</td>
<td>27.161 ± 40.326</td>
<td>0.501</td>
</tr>
</tbody>
</table>

1 All analyses were conducted with multivariable linear regression with the statistical covariates listed in the table (n = 2075). Statistical significance was determined after correction for multiple comparisons, or *P* ≤ 0.004.
2 As the marker minor allele frequency fell below 20%, the rare genotype was combined with the intermediate genotype.
3 *P* values of nominal significance.
Eating occasions (no./d)

Gene | Single nucleotide polymorphism | Minor allele | $\beta \pm SE$ | $P$ value | $\beta \pm SE$ | $P$ value
--- | --- | --- | --- | --- | --- | ---
TNNI3K | rs1514176 | G | $-0.030 \pm 0.037$ | 0.414 | $-0.041 \pm 0.036$ | 0.255
INSIG2 | rs7566605 | C | $-0.042 \pm 0.038$ | 0.263 | $-0.037 \pm 0.037$ | 0.317
PPARG | rs1801282 | G$^2$ | $0.153 \pm 0.074$ | 0.038$^4$ | $0.128 \pm 0.073$ | 0.080
NISCH-STABI | rs4687617 | G | $0.027 \pm 0.082$ | 0.742 | $0.006 \pm 0.081$ | 0.945
BDNF | rs10767664 | T$^2$ | $-0.024 \pm 0.056$ | 0.661 | $0.003 \pm 0.055$ | 0.951
BDNF | rs6265 | A$^2$ | $-0.005 \pm 0.058$ | 0.927 | $0.023 \pm 0.057$ | 0.682
BDNF | rs1401635 | C | $-0.054 \pm 0.039$ | 0.160 | $-0.048 \pm 0.038$ | 0.205
MTIF3 | rs7988412 | A | $0.028 \pm 0.054$ | 0.601 | $0.026 \pm 0.053$ | 0.623
MAP2K5 | rs2241420 | A | $-0.023 \pm 0.037$ | 0.524 | $-0.026 \pm 0.036$ | 0.470
SH2B1 | rs4788099 | G$^2$ | $-0.003 \pm 0.038$ | 0.936 | $-0.011 \pm 0.037$ | 0.758
FTO | rs1421085 | C | $0.125 \pm 0.039$ | 0.001$^4$ | $0.109 \pm 0.038$ | 0.004$^4$
FTO | rs3751812 | A | $0.096 \pm 0.039$ | 0.014$^4$ | $0.081 \pm 0.038$ | 0.034$^4$
FTO | rs9922708 | A | $0.077 \pm 0.037$ | 0.039$^4$ | $0.063 \pm 0.037$ | 0.087
FTO | rs9939609 | A | $0.087 \pm 0.037$ | 0.015$^4$ | $0.075 \pm 0.036$ | 0.037$^4$
MC4R | rs17782313 | C | $-0.113 \pm 0.041$ | 0.006$^4$ | $-0.112 \pm 0.041$ | 0.006$^4$
QPCTL/GIPR | rs11672660 | A$^2$ | $0.154 \pm 0.054$ | 0.005$^4$ | $0.146 \pm 0.053$ | 0.006$^4$

$^1$ All analyses were conducted with multivariable linear regression with the statistical covariates listed in the table ($n = 2075$).
$^2$ Because the marker minor allele frequency fell below 20%, the rare genotype was combined with the intermediate genotype.
$^3$ $P$ values of nominal significance.
$^4$ Statistical significance after correction for multiple comparisons, or $P \leq 0.004$.

statistical adjustment for total caloric intake did not substantially alter the association of $SH2B1$ rs4788099 with more servings per day of dairy products ($P = 0.002$). No associations were observed with servings of vegetables (PFG2) or fruit (PFG3) before or after statistical adjustment for total caloric intake.

**DISCUSSION**

GWAS have been successful in identifying common genetic variants that are associated with obesity. However, the mechanisms through which these polymorphisms affect obesity remain unclear. Here we report that select obesity genetic risk markers may affect meal patterning and servings per day within specific Food Guide Pyramid food groups, such as dairy products, as measured by an FFQ.

Obesity risk alleles at $FTO$ rs1421085 were significantly associated with a greater number of meals and snacks per day, with nominal associations with greater total caloric intake, greater percentage of energy from fat, and more servings of fats, oils, and sweets. The association of $FTO$ obesity risk SNPs with a greater number of eating occasions was not substantially diminished by adjustment for total caloric intake. The overall picture suggests that variation in $FTO$ may bias meal patterning and perhaps total caloric intake and consumption of sweet or high-fat foods.

Because rs1421085 is in high linkage disequilibrium with rs9939609, our results are largely consistent with prior research in children, which indicates that $FTO$ rs9939609 is associated with a preference for energy-dense food (13), greater consumption of fat and calories (14), consumption of palatable food after having eaten a meal (37), and reduced satiety (15). However, we tested rs9939609 directly in our analyses and found more consistent associations with rs1421085. Previously in adults, no association between $FTO$ rs9939609 and caloric intake or percentage of energy from fat intake has been found (17, 20). Although this suggests that rs1421085 may better capture the effect of this region on dietary intake, the functional significance of this locus in obesity and the role of both rs1421085 and rs9939609 remain to be determined (38, 39).

Risk alleles at $BDNF$ rs6265 and rs10767664 predicted a pattern of dietary variables, including servings of meats, eggs, nuts, and beans and servings of dairy products with nominal associations with total caloric intake; servings of breads, cereals, rice, and pasta; and servings of sweets and fats. $BDNF$ and its primary receptor TrKB are expressed key brain regions of the hypothalamus and dorsal vagal complex related to body weight and energy homeostasis (40–44). Targeted disruption of $BDNF$ in transgenic models results in hyperphagia and obesity (45–49). $BDNF$ rs6265 leads to a valine-to-methionine substitution at position 66 (Val66Met) in the prodomain of the gene. Moreover, at least one case study also links rare mutations in $BDNF$ to severe obesity in an 8-y-old girl (50). The associations with servings within the various food groups were diminished on statistical adjustment for total caloric intake, which suggests that the primary effect was on caloric intake but that the total was achieved through servings with the associated food groups.

The risk allele at rs1514176, an intronic SNP located within $TNNI3K$, was significantly associated with a lower percentage of energy from protein and nominally associated with more daily servings of fats, oils, and sweets. $TNNI3K$ is a cardiac troponin-interacting kinase expressed primarily in the heart (51). Little is currently known about the mechanisms through which this gene
region influences obesity. Because protein is considered to be the most satiating macronutrient (52), diets lower in protein intake may actually contribute to increased overall energy intake as a result of reduced overall feelings of fullness and thereby to a greater degree of positive energy balance.

The risk allele at rs4780099 in the SH2B1 region was significantly associated with more daily servings of dairy products. SH2B1 is involved in leptin signaling. Deletions in this region, including SH2B1, have been associated with early-onset obesity (53). Whereas consumption of more daily servings of dairy products, particularly lower-fat dairy products, has been associated with lower adiposity (54), the food group in this investigation contains both low- and high-fat products. Thus, this relation may be driven by greater intake of high-fat dairy product servings.

It was interesting to note that the number of eating episodes was among the dietary phenotypes most strongly associated with genetic polymorphisms. The relation of number of eating occasions and risk of overweight has been debated. In laboratory studies, it has been shown in some but not all (55, 56) studies that spreading the same total caloric intake over a greater number of meals per day may better control appetite. In free-living populations, it is quite plausible that extra eating occasions could promote greater caloric intake and weight gain; however, the data appear mixed. Certain studies find that a greater number of meals per day is associated with lower body weight (57), whereas others find the opposite effect or no association (58, 59).

The strengths of this study included a large ethnically diverse sample inclusive of men and women, control for population stratifications, use of a well-validated FFQ, and genotyping of many genetic markers previously associated with obesity. Limitations include basing conclusions on a select cohort of individuals (overweight or obese with type 2 diabetes) and, therefore, conclusions may not be generalizable. Similar to other genetic association studies, the size of the cohort may have prevented us from detecting more modest effects. Although we observed consistent patterns of association, many of the associations were of nominal significance and replication in an independent cohort should be conducted to support or refute these results.

Our study was also cross-sectional in design, which prevented us from determining the time precedence of genetic associations with diet and obesity (eg, whether genetic associations with diet preceded genetic associations with obesity, or vice versa). For example, if diet is a mechanism through which these SNPs increase risk of obesity, it would be expected that associations with diet would precede associations with obesity. It is also possible that people with larger body sizes eat more to maintain their weight, in which case genetic associations with obesity would be expected to precede those with diet. These are important questions for future research with longitudinal modeling.

The measurement of dietary intake also has limitations. Whereas the USDA’s 5-pass method, 24-h dietary recall is currently considered to be the gold standard of dietary assessment (60, 61), FFQs are usually used in large samples to assess dietary intake over a specified time period because of their low cost, ease of administration (62), and prior validation against the 24-h dietary recall (26). Because FFQs are conducted by self-report, it is recognized that underreporting of dietary intake is common, particularly in individuals who are overweight (63–66), have diabetes (66), and are wanting to reduce their weight (64). It is plausible that a tendency to underreport dietary intake may have influenced our results. Given the variability of body weight in the sample, it is also possible that systematic underreporting may have occurred, such that greater underreporting occurred among those with the highest BMI. Moreover, physical activity and other factors that determine energy expenditure and thereby energy intake may influence the correlation between weight and caloric intake. Nonetheless, underreporting should have restricted the range of self-reported dietary intake in our sample, rendering the detection of significant associations more difficult. Thus, the identification of genetic associations with dietary intake via FFQs suggests that the strength of these associations may improve with more precise measurement.

In summary, our results suggest that select obesity genetic risk markers—particularly markers within FTO, BDNF, TNNI3, and SH2B1—predict a pattern of obesogenic dietary intake, including a higher number of eating occasions per day and more servings from calorically dense food groups. If replicated, these results could inform the mechanisms through which these genetic markers are associated with adiposity.

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